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Introduction

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Abstract

The phosphoinositide-3-kinase (PI3K) family of lipid kinases has been well conserved from yeast to mammals. In this evolutionary perspective on the PI3K family, we discuss the prototypical properties of PI3Ks: 1) the utilization of sparse but specifically localized lipid substrates; 2) the nucleation signaling complexes at membrane-targeted sites; and 3) the integration of intracellular signaling with extracellular cues. Together, these three core properties serve to establish order within the entropic environment of the cell. Many human diseases, including cancer and diabetes, are the direct result of loss or defects in one or more of these core properties, putting much hope in the clinical use of PI3K inhibitors singly and in combination to restore order within diseased tissues.

1 Establishing Order Within the Cell

The lipid kinase activity of phosphoinositide 3-kinase (PI3K) has been evolutionarily conserved from yeast to mammals and has evolved from a simple means of sorting vacuolar proteins to nucleating large signaling complexes that regulate growth, metabolism and survival (Engelman et al. 2006). Here, we reflect on the unique properties of PI3Ks that explain the diverse roles that these enzymes play in cellular regulation and their relevance in multiple human diseases.

A typical mammalian cell is composed of approximately 70% water and 20% proteins. In their textbook example, Lodish and colleagues estimate that for a hepatocyte this translates into roughly 8×10^9 protein molecules, most of which are randomly diffusing within a chaotic 15-μm^3 space (Lodish et al. 2000). In such a disordered environment, order and directionality must be established to successfully transmit growth and survival signals, for example from a membrane-anchored growth factor receptor to a transcription factor in the nucleus. Perhaps the most valuable and thus conserved property of PI3K is the ability to impose such order in a highly entropic environment.

The core properties that allow PI3K to carry out this function have been conserved from unicellular to multicellular organisms. These include (1) having low abundant but highly specific lipid substrates and products; (2) generating membrane-anchored products that nucleate signaling complexes at targeted sites; and (3) having the ability to associate with membrane-bound proteins that sense extracellular stimuli. Over the course of evolution, higher organisms have evolved several classes of PI3Ks that utilize these prototypical properties to regulate a wide range of functions ranging from directional motility to metabolism, growth, and survival. Importantly, it is also the loss of these core properties that result in aberrant signaling and disease.

2 Phosphatidylinositol and Phosphoinositides as Ideal Substrates

Evolving biological systems require simplicity that will not convolute cellular communication or waste resources. Yet there must be enough variability in the system to

allow for diversification and selection. Following this model, PI3K has only three lipid substrates: phosphatidylinositol (PtdIns) and two of its phosphoinositide derivatives, PI-4-P and PI-4, 5-P_2. Additionally, these substrates are present at low levels within the cell. While only 5% of the mass of a mammalian cell is comprised of lipids, only 4% of total lipids are PtdIns and less than 1% of total PtnIns is phosphorylated. Importantly, the PI3K products make up only about 1% of the total phosphorylated forms of PtdIns (Mulgrew-Nesbitt et al. 2006). This extreme low abundance of PI3K lipid products ensures that PI3K signaling is deliberate, dynamic, non-promiscuous, and exquisitely localized.

Yet, despite the scarcity of PtdIns in the cell, the inositol head group contains five free hydroxyl groups that could potentially be phosphorylated to generate variability in the phosphoinositide pool. Three of the five hydroxyl groups (D3, D4, and D5 positions) are phosphorylated alone or in combination, yielding seven phosphoinositides, each with unique stereospecificity and charge. At least 10 discreet protein domains have independently evolved the ability to bind one or more phosphoinositides and have been identified in hundreds of proteins across numerous species (Lemmon 2008; DiNitto et al. 2003). Thus, by modifying a single lipid substrate, the phosphoinositide kinases have evolved the unique ability to regulate numerous proteins while carefully preserving specificity.

3 Nucleating a Protein Complex at a Target Location

The most ancient role of PI3K in unicellular organisms remains arguably its most relevant role in multicellular organisms. This is the role of nucleating a protein complex at a target location within the cell. *Saccharomyces cerevisiae* expresses the most primordial PI3K, the class III Vps34, which generates PI-3-P at sorting endosomes. Proteins containing FYVE domains bind to PI-3-P and form complexes that regulate vacuolar protein sorting (Burd and Emr 1998). The generation of PI-3-P specifically at sorting endosomes ensures that the protein-sorting complexes are carefully localized to this compartment. Proper localization of protein complexes is also critical for directional movement in another unicellular organism, *Dictyostelium discoideum*. The generation of PI-3, 4, 5-P_3 by class I PI3K at the cell's leading edge recruits PH domain containing proteins such as CRAC and AKT that rearrange the cytoskeleton for directed movement towards shallow chemoattractant gradients (Parent et al. 1998; Meili et al. 1999).

The need for proper protein localization for the most basic functions in unicellular organisms suggests that this was the original function of PI3K. Multicellular organisms have conserved this property by utilizing localized phosphoinositides to regulate cellular polarity and migration, particularly in epithelial cells, neutrophils, and macrophages (Gassama-Diagne et al. 2006; Fruman and Bismuth 2009). However, the utility of this enzyme in multicellular organisms extends into far more complex realms of signaling that nevertheless hinge on the ability to nucleate large signaling complexes at cellular membranes.

4 Coupling PI3K Activity to Extracellular Cues

The evolution towards multicellularity was accompanied by the emergence of two additional classes of PI3K. In addition to class III, class I and II PI3Ks are found in *Caenorhabditis elegans*, *Drosophila melanogaster*, and all vertebrates, suggesting that these later evolving classes specialize in mediating cell–cell communication. Extracellular sensing in unicellular organisms is essentially a survey of the local nutrient landscape, which informs the cell whether or not to grow and proliferate. In multicellular organisms, numerous extracellular stimuli instruct cells not just to grow and proliferate, but also to migrate to new location, to activate survival mechanisms, and to alter gene expression programs to control metabolic needs and other specialized functions of differentiated tissues.

Class I and II PI3Ks evolved to cope with these new and complex demands by targeting the assembly of various protein complexes directly downstream of membrane-bound receptors, thereby integrating intracellular signaling with extracellular cues. Class I PI3Ks are targeted to active receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs). For class IA PI3Ks, localization to RTKs or adaptors downstream of RTKs is facilitated by conserved SH2 domains in the p85 family of regulatory subunits, which bind to Tyr-phosphorylated Y-X-X-M motifs on activated RTKs or adaptor molecules. The class IB PI3K, p110γ, localizes to GPCRs through the interaction of a regulatory subunit, p101 or p84, with Gβγ subunits. The class IA PI3K, p110β can bind to both RTKs and to Gβγ subunits of GPCRs and appears to act as an integrator of signaling through both pathways. Class II PI3Ks are less well studied, but can be activated by RTKs such as the epidermal growth receptor (EGFR) and insulin receptor and play an important role in clathrin-mediated vessel trafficking (Williams et al. 2009).

Due to their specialized role in interpreting extracellular cues, class I PI3Ks have been most extensively studied. It is the only class that generates PI-3, 4, 5-P_3, a potent second messenger, from PI-4, 5-P_2 in the membrane. High local concentrations of PI-3, 4, 5-P_3 in the membrane colocalize signaling molecules containing PH domains, which bind PI-3, 4, 5-P_3 with high specificity. Some of these molecules include guanine nucleotide exchange factors (GEF) such as GRP1, as well as kinases such as the Bruton tyrosine kinase (BTK), other members of the Tec family of non-receptor tyrosine kinases, and the serine/threonine kinases AKT (also known as PKB) and phosphoinositide-dependent kinase 1 (PDK1) (Cantley 2002). Importantly, localized pools of PI-3, 4, 5-P_3 are generated transiently, limited by the local availability of PI-4, 5-P_2 and by rapid degradation due to the presence of nearby phosphoinositide phosphatases. Three families of phosphoinositide phosphatases are important in modulating class I PI3K signaling: (1) PTEN dephosphorylates the 3′ position of PI-3, 4, 5-P_3 to regenerate PI-4, 5-P_2; (2) SHIP family members dephosphorylate the 5′ position of PI-3, 4, 5-P_3 to generate PI-3, 4-P_2; and (3) INPP4A/4B family members dephosphorylate the 4′ position of PI-3, 4-P_2 to generate PI-3-P. Ultimately, disengagement of PI3K from receptors due to various negative feedback loops terminates signaling. Temporal and spatial regulation of PI-3, 4, 5-P_3 (as well as PI-3, 4-P_2) production is unique to class I PI3Ks and highlights the importance of negatively regulating this complex pathway for receptor-mediated signaling (Lemmon 2008).

One PI-3, 4, 5-P_3 binder in particular, AKT, is responsible for much of this complexity. The PH domain of AKT can bind to both PI-3, 4, 5-P_3 and PI-3, 4-P_2, and both lipids appear to contribute to AKT recruitment to membranes (Franke et al. 1997), as well as recruitment of the upstream activating kinase, PDK1. There are more than 100 reported substrates for AKT, identified under varying degrees of stringency (Manning and Cantley 2007). Canonical AKT substrates contain R-X-R-X-X-S/T motifs, and phosphorylation on serine or threonine residues initiates a complex cascade of signaling events in the cytosol and nucleus. Briefly, AKT promotes proliferation through the inhibitory phosphorylation of FOXO transcription factors and GSK3, promotes protein synthesis by phosphorylating TSC2 and PRAS40, promotes cell survival by phosphorylating BAD and MDM2, and induces glucose uptake in GLUT4-containing cells through phosphorylation of AS160 (Manning and Cantley 2007). Interestingly, by comparing the number, specificity, and location of PI3K and AKT substrates, it is clear that these two well-conserved enzymes serve non-redundant functions as initiator and effector kinases, so to speak. Given the breadth of their cooperative actions, it is important to note that these two kinases are regulated by multiple feedback mechanisms to ensure that the system resets to basal levels following acute cell stimulation by growth factors (Engelman et al. 2006).

5 Disease Implications

As discussed above, though the responsibilities of PI3K in the cell have vastly increased over the course of evolution, its core properties have remained unchanged. Using low abundance, membrane-anchored lipids to organize signaling complexes downstream of extracellular cues persists as an optimal mode of transmitting signals within the cell. It is, thus, not surprising that alterations in these core properties result in evolutionarily unfavorable signaling and disease. Alterations in the PI3K pathway account for at least 30% of all human cancers and have been implicated in type-2 diabetes. To conclude, we briefly review how genetic alterations in PI3K pathway components unhinge the core properties of PI3K.

In normal cells, the low abundance of phosphoinositides ensures deliberate and targeted signaling downstream of PI3K activation. However, through loss of the PI-3, 4, 5-P_3 phosphatase, PTEN, or oncogenic hotspot mutations in p110α that confer constitutive kinase activity, PI-3, 4, 5-P_3 levels and cellular distribution increase dramatically, resulting in promiscuous and prolonged downstream signaling that contributes to tumorigenesis (Engelman et al. 2006). Recent work has also shown that other components of the PI3K signaling network that influence the phosphoinositide pool, including p85α and INPP4B, are frequently mutated or deleted in human cancers (Cancer Genome Atlas Research Network 2008; Gewinner et al. 2009).

The nucleation of protein complexes not only organizes signaling molecules to one location, but the systematic assembly of the complex ensures that signaling only occurs when all components are primed and ready. This system of checks and balances is disrupted, for example, in tumors with E17K mutations in AKT or p110α hotspot mutations (Carpten et al. 2007). Through enhanced enzymatic activity and membrane localization, cells bearing these mutations are no longer dependent on the step-wise assembly of the signaling complex, and can independently (over)activate downstream pathways that can lead to hyperproliferation.

Lastly, by coupling PI3K activity to activated extracellular receptors in normal cells, intracellular signaling is in harmony with extracellular conditions. However, in many tumors with RTK mutations or amplifications, such as in ERBB1 and ERBB2, extracellular conditions are exaggerated and can result in unsustainable PI3K signaling. In the other extreme, when PI3K activity is uncoupled from RTK signaling, such as in models of insulin resistance where PI3K is insensitive to insulin receptor activation, type-2 diabetes can arise (Engelman et al. 2006).

Given the large role PI3K plays in tumorigenesis and in the activation of macrophages and lymphocytes, there has been a profound effort by pharmaceutical companies to develop targeted inhibitors of this pathway for treating cancers and for suppressing immune responses. Several PI3K inhibitors, with varying specificities for submembers of the family, have shown great promise in pre-clinical cancer models and are currently in phase I/II clinical trials. AKT catalytic site inhibitors have also entered phase I clinical trials. It is now clear from extensive sequencing of genes from primary human cancers that mutations in the PI3K pathway are very frequent, but that they are invariably combined with mutations in other pathways. Thus, while there is much excitement about the PI3K and AKT inhibitors, it is likely that these compounds will need to be combined with other drugs that target other pathways activated in the same tumors in order to be effective. There is much hope that future “personalized” clinical trials that focus on matching drugs to mutational events in individual tumors will further validate targeted therapy and provide a logical path for conquering the myriad of cancers that have evaded more conventional cancer therapies over

the past 40 years. It is likely that PI3K inhibitors will be a significant addition to the arsenal needed for this approach.

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